Apidologie 38 (2007) 247–258
© INRA/DIB-AGIB/ EDP Sciences, 2007
DOI: 10.1051/apido:2007006

Available online at:
www.apidologie.org

Original article

Morphological and molecular characters reveal differentiation in a Neotropical social bee, *Melipona beecheii* (Apidae: Meliponini)*

José Javier G. QUEZADA-EUÁN[a], Robert J. PAXTON[b], Kellie A. PALMER[c], William de J. May ITZÁ[a], Wee Tek TAY[d], Benjamin P. OLDROYD[c]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Apdo. postal 4-116, Mérida Yucatán 97100, Mexico
[b] School of Biological Sciences, Queen's University Belfast, UK
[c] School of Biological Sciences A12, University of Sydney, NSW 2006, Australia
[d] ARC Department of Genetics Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, Victoria 3010, Australia

Received 23 February 2006 – Revised 21 September 2006 – Accepted 8 October 2006

Abstract – Morphometrics and DNA microsatellites were used to analyse the genetic structure of populations of the stingless bee *M. beecheii* from two extremes of its geographic range. The results showed that populations from Costa Rica and Yucatan exhibit substantial phenotypic and molecular differentiation. Bees from Yucatan were smaller and paler than those from Costa Rica. The value of multilocus $F_{ST} = 0.280$ ($P < 0.001$) confirmed that there were significant molecular genetic differences between the two populations. Populations showed significant deviation from Hardy Weinberg equilibrium and the values of F_{IS} (the inbreeding coefficient) were positive for Costa Rica = 0.416 and the Yucatan Peninsula = 0.193, indicating a lack of heterozygotes in both populations possibly due to inbreeding. The DNA sequence of 678 bp of the mitochondrial gene COI differed between populations by 1.2%. The results of this study should be considered in conservation programmes, particularly with regard to the movement of colonies between regions.

stingless bee / *Melipona* / population analysis / Yucatan / Costa Rica / genetic diversity

1. INTRODUCTION

The Meliponini or stingless bees (Hymenoptera: Apidae) are amongst the most abundant and ecologically important social invertebrates in tropical communities due to their keystone role in the pollination of large numbers of wild plants and crops (Roubik, 1989). Compared to their sister group the honey bees (Apini) that are native to much of the tropical and temperate Old World (Ruttner, 1988), the 400 plus species of pantropical Meliponini have been little studied, the result being that the classification of the group is still largely unresolved (Camargo et al., 1988; Michener, 2000). Moreover, few extensive analyses have been conducted on the population structure of stingless bee species (cf. Waldschmidt et al., 2002; Castanheira and Contel, 2005). Thorough analyses of other members of the family Apidae (e.g. the honey bees *Apis mellifera, Apis cerana* and *Apis florea* plus the bumble bees *Bombus* spp., see Radloff et al., 2005; Hepburn et al., 2001, 2005; Estoup et al., 1996; Widmer et al., 1998, Widmer and Schmid-Hempel, 1999) have revealed patterns of subtle differentiation amongst populations.

Corresponding author: J.J.G. Quezada-Euán, qeuan@tunku.uady.mx
* Manuscript editor: Walter S. Sheppard

The Yucatan Peninsula of southern Mexico was a major centre for stingless bee keeping and for trade in their products to other sites in Mesoamerica ca. 1000–3000 years ago (Quezada-Euán et al., 2001). The species most intensively exploited by the peoples of the Yucatan Peninsula then and now, the Maya Indians, was *Melipona beecheii* Bennett, a species which was then very abundant. Nowadays stingless beekeeping is at the verge of disappearing from the region, with potentially many negative consequences such as the loss of genetic variability in managed colonies within apiaries (termed meliponarios) (Quezada-Euán et al., 2001). The result is that nowadays, *Melipona beecheii* is listed as an endangered native bee species (Kerr, 2002), whose numbers have steadily declined, both in terms of managed and wild colonies (Quezada-Euán et al., 2001).

The geographic range of *M. beecheii* covers a large area from southern Mexico in the north down to Costa Rica in the south (Biesmeijer, 1997; Ayala, 1999). In Mexico, *M. beecheii* is reported from the Yucatan Peninsula and along both the tropical Gulf of Mexico and the Pacific coasts (Ayala, 1999). Such an extensive distribution encompasses diverse environmental conditions and associated habitat types. Therefore, it is possible that many locally adapted ecotypes have arisen (Camargo et al., 1988).

Genetic analysis of populations has been greatly enhanced with the progress in molecular techniques. Nowadays, it is possible to combine information derived from numerical taxonomy and molecular methods to provide a better profile of the population differentiation of a species. For example, although not always in total agreement, both morphological and molecular genetic tools have proven to be powerful methods with which to establish the genetic structure in populations of other bee species such as *Apis mellifera* (Ruttner, 1988; Estoup et al., 1996; Franck et al., 2001) and *Bombus* spp. (Estoup et al., 1996; Widmer et al., 1998; Widmer and Schmid-Hempel, 1999).

In this paper, we analyse the structure of populations of *M. beecheii* collected at two extremes of its geographic range, combining morphometrics, microsatellites and mitochondrial DNA sequences to address the following questions: (1) what is the degree of phenotypic and genetic differentiation between populations, (2) is there agreement between morphometric and molecular data, and lastly (3) since *M. beecheii* populations from the Yucatan Peninsula have been under management for centuries, we also tested if substantial inbreeding may have occurred in them.

Figure 1. Map of *M. beecheii* collection sites in the Yucatan Peninsula and Costa Rica. The collection sites in the three states of the Yucatan Peninsula are symbolised with triangles (Yucatan), diamonds (Campeche) and circles (Quintana Roo). The sampling site in Costa Rica is symbolized with a hexagon.

2. MATERIALS AND METHODS

2.1. Sample collection

Bees were collected in 1998 from seven sites across the Yucatan Peninsula (65 colonies – at altitudes between 12 to 50 m asl.) and one site from Guanacaste, Costa Rica (15 colonies at an altitude of de 200 m asl.; Fig. 1). Each sample consisted of ca. 15 young *M. beecheii* workers collected from the central part of one colony. All samples were from managed colonies and were preserved in ethanol at –20 °C for the same length of time until morphometric and molecular analyses were conducted.

2.2. Morphometric analyses

The aim of the study was to establish differences between the Yucatan Peninsula and Costa Rican populations. But since the samples from the Yucatan Peninsula were collected in 7 localities and the ones from Costa Rica only in one, it was necessary first to establish if there was homogeneity in the Yucatan Peninsula samples. For this, the Yucatan Peninsula samples were divided into 3 groups defined by the Mexican states from which they were collected: Campeche (15 samples- 1 inland locality); Quintana Roo (20 samples- 3 coastal localities) and Yucatán (30 samples- 3 inland localities) to analyse the variation amongst them (Fig. 1).

Eleven morphometric characters from the head, wings and legs were measured in 10–12 workers from each colony. The morphometric characters were chosen in accordance with Hartfelder and Engels (1992) and Diniz-Filho and Pignata (1994). The degree of maculation of the head (HM) was also considered due to the variation in it already observed between populations (Camargo et al., 1988; Ayala, 1999). The head, right forewing, right hindwing and right posterior leg of each worker bee were dissected with the help of a stereomicroscope (10× magnification) and were mounted on 35 mm photographic film slides. The head length (HL); head width (HW); clypeus width (CW); forewing length (WL); forewing width (WW); hind wing length (HWL); hind wing width (HWW); femur length (FEL); tibia length (TIL); tibia width (TIW) and basitarsus width (BAW) were measured to an accuracy of 0.001 mm by means of an inverted dissecting microscope that projected the image of the structures onto a digitizer pad. The distances between points on the digitizer pad were converted to micrometers by means of the program AFUSDA7 (Rubink, unpubl. data). The degree of head maculation was calculated as the area of the clypeus and the supreaclypeal and malar areas covered by yellow marks. We assigned each worker to one of 3 main types of head maculation: (1) scarce, yellow marks covering less than 10% of the clypeus and the supraclypeal area; hardly visible in the malar area around the eyes; (2) medium, yellow marks between 10–50% of the clypeus and the supraclypeal area; extending as thin lines in the malar area around the eyes; (3) intense, yellow marks covering more than 50% of the clypeus and the supraclypeal area, extending in the form of a broad line in the malar area around the eyes (Fig. 2).

After measuring all characters, they were compared among the three Yucatan Peninsula states of Mexico (Campeche, Yucatan and Quintana Roo) using ANOVA and Tukey's test for a posteriori comparison among means.

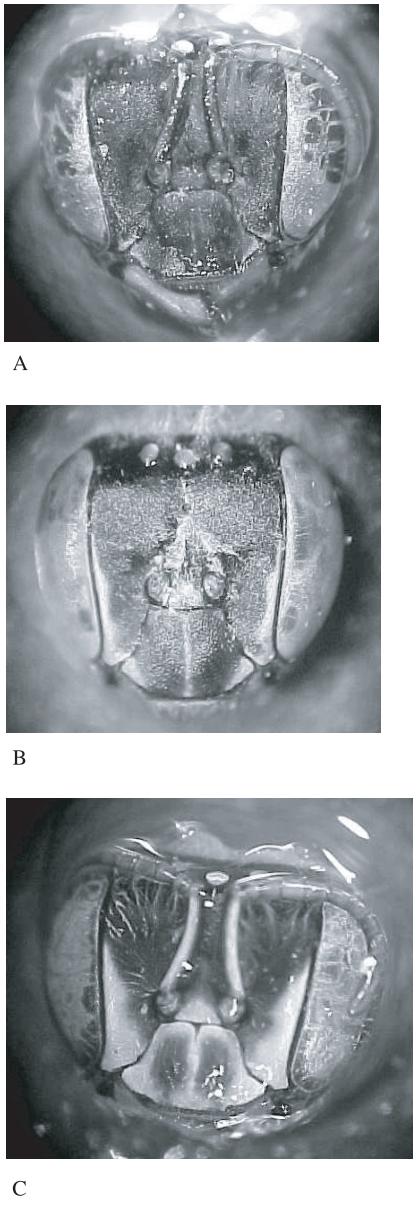

Figure 2. Degree of maculation on the head of *M. beecheii*: (A) scarce (typical in specimens from Costa Rica); (B) medium; (C) intense (typical in specimens from the Yucatan Peninsula).

Differences in head maculation were compared between the three states by means of a contingency *G* test. Then, a Principal Component Analysis (PCA

using a correlation matrix) was performed on all log-transformed metric characters (Wiley, 1981). Eigenvalues (these define the amount of total variation that is displayed on the PC axes) and the percentage of the variance were obtained only for the first 4 components, since they usually include the largest amount of variation in the sample (Willey, 1981). Character loadings were calculated for each of the four components in order to obtain the association (positive or negative) of each character with a component; only associations more extreme in absolute value > 0.5 were considered. After homogeneity had been demonstrated in the samples from the Yucatan Peninsula, we proceeded with the comparison between the two populations, Costa Rica and the Yucatan Peninsula of Mexico. The same analyses above were conducted to compare the 65 samples from the Yucatan Peninsula with the 15 samples from Costa Rica. Additionally, for this part of the study, colony Principal Component Scores (PCS) were obtained by multiplying the character coefficients for the four components by their mean value for each colony. Colony PCS from the Yucatan Peninsula and Costa Rica were compared by means of ANOVA and were plotted orthogonally against the axes of components 1 and 2 to obtain a comparative spatial distribution of the two populations.

2.3. Molecular genetic analyses

Total DNA was extracted from the thorax of one bee per colony, using a high salt protocol (Paxton et al., 1999). Six microsatellite loci (T4, T7, Mbi11, Mb201, B116 and B124) were analysed using the primers and amplification characteristics described in Estoup et al. (1993, 1995); Paxton et al. (1999) and Peters et al. (1999). Additionally, part of the mitochondrial gene COI was sequenced in 2 individuals from Costa Rica and 2 from Yucatán (location Mérida) randomly taken from the samples above, and all four from different colonies using primers Pat and Jerry and the PCR conditions as described in Simon et al. (1994). The sequences were aligned to a sequence from *M. bicolor* in GenBank (GenBank Accession Number AF466146; Sylvester and Arias, unpubl. data). The analysis of microsatellites was conducted at the University of Sydney and the University of Yucatan whilst the COI analysis was conducted at Queen's University Belfast.

Linkage of microsatellite loci was tested using the software package GENEPOP version 3.1 (Raymond and Rousset, 1995). Allele frequencies, observed and Nei's (1978) unbiased estimates of expected heterozygosities of each microstellite locus were estimated for each population using the FSTAT package (version 1.7 Goudet, 1996). The effective number of alleles (n_a) was computed as $n_a = 1/\Sigma p_i^2$ where p_i is the frequency of the ith allele. Departures from Hardy-Weinberg equilibrium for each locus and population were carried out by exact tests, as implemented in GENEPOP using a Markov chain approach.

The unbiased multilocus estimate of F_{ST} between populations was used to determine the significance of genetic differentiation (Weir and Cockerham, 1984) using the package FSTAT (Goudet, 1996), which was also used to calculate the inbreeding coefficient F_{IS} and its confidence intervals. We assembled contigs using Staden (Staden et al., 2000) and employed ClustalX to align sequences (Thompson et al., 1994) using default settings.

3. RESULTS

3.1. Morphometric analyses

ANOVA of meristic characters from samples within the Yucatan Peninsula showed that there were no significant differences for 10 morphometric characters except for femur length, which was slightly longer in samples from Quintana Roo (2.419 ± 0.032) compared to Yucatan (2.335 ± 0.036) and Campeche (2.313 ± 0.053) (ANOVA $P > 0.01$). However, the PCA revealed no statistical differences in the first 4 components of the analysis ($P > 0$ 0.01). Facial colouration did not vary among samples from the Yucatan Peninsula ($P > 0.05$). We concluded that the samples of the Yucatan Peninsula were homogeneous and comparison with bees from Costa Rica could proceed without including undetected within-region differences.

There were significant differences between the *M. beecheii* populations from the Yucatan Peninsula and Costa Rica in all eleven morphometric characters (Tab. I). In all characters analysed, the Yucatan Peninsula bees were significantly smaller than those from Costa Rica. The characters with the largest variation between the means of the two groups were CW

Table I. Univariate comparison of eleven morphometric characters in populations of *M. beecheii* from the Yucatan Peninsula and Costa Rica. Mean ± standard deviation.

Character (in mm)	Yucatan Peninsula (n = 65)	Costa Rica (n = 15)
Head width (HW)	3.728 ± 0.08[a]	3.898 ± 0.067[b]
Head length (HL)	2.565 ± 0.045[a]	2.588 ± 0.051[b]
Clypeus width (CW)	1.639 ± 0.042[a]	1.771 ± 0.059[b]
Forewing length (FWL)	7.102 ± 0.084[a]	7.793 ± 0.128[b]
Forewing width (FWW)	2.547 ± 0.080[a]	2.759 ± 0.045[b]
Hind wing length (HWL)	4.510 ± 0.079[a]	5.545 ± 0.083[b]
Hind wing width (HWW)	1.445 ± 0.044[a]	1.539 ± 0.037[b]
Femur length (FL)	2.355 ± 0.055[a]	2.535 ± 0.044[b]
Tibia length (TL)	2.783 ± 0.065[a]	3.111 ± 0.112[b]
Tibia width (TW)	1.178 ± 0.023[a]	1.213 ± 0.041[b]
Basitarsus width (BW)	0.838 ± 0.032[a]	0.891 ± 0.021[b]

Different letters within a row indicate significant differences among means (ANOVA, $P < 0.05$).

Table II. Eigenvalues and percentage of variance explained by the first four components in a PCA of populations of *M. beecheii* from the Yucatan Peninsula and Costa Rica.

Component	Eigenvalues	Percentage of variance	Cumulative percentage
1	5.321	42.15	42.15
2	1.857	17.60	59.75
3	1.251	9.42	69.17
4	0.991	6.85	75.97

Table III. Colony PC scores for the first four components of a PCA between populations of *M. beecheii* from the Yucatan Peninsula and Costa Rica. The first figure in each column is the population mean and the second is the standard deviation.

Character	Yucatan Peninsula (n = 65)	Costa Rica (n = 15)
Component 1	–0.124 ± 0.120[a]	2.982 ± 0.033[b]
Component 2	–0.090 ± 0.065[a]	1.686 ± 0.112[b]
Component 3	0.031 ± 0.012[a]	0.675 ± 0.051[b]
Component 4	0.068 ± 0.024[a]	0.250 ± 0.020[b]

Means followed by different letters within a row differ at $P < 0.05$.

(8%), WL (9%), WW (7%) and FL (9%). The largest difference between the character means of both groups was for TL at 10% (Tab. I).

The degree of head maculation was markedly different between the bees from Costa Rica and the bees from the Yucatan Peninsula (G test $\chi^2_{(0.05,2)} = 34.14$, $P < 0.01$; see Fig. 2). Yellow marks were almost absent in the face of Costa Rican bees and, when present, scarcely covered the edge of the clypeus and malar area whilst they were highly conspicuous on the clypeus and a large portion of the malar area in all Yucatan Peninsula bees.

For the PCA, the Kaiser Meyer Olkin measure of sampling adequacy was 0.85 and Bartlett's test of sphericity was 567.15 ($P < 0.01$), indicating that the correlation matrix was not an identity matrix. The results from the former two tests demonstrate that the data were suitable for PCA. The first four components of the PCA included 75.9% of the variance in the morphometric characters, and components 1 and 2 alone comprised 59% (Tab. II).

The character loadings for each component showed that HW, HWL, HWW and TW were not closely correlated with component 1 whilst all other characters were closely correlated. However, since all variables were positively correlated with this component, it provides evidence that component 1 is related to body size. Thus, 42% of the variance in the analysed populations was due to size differences (Tab. II).

In contrast components 2 to 4 included characters negatively correlated with their respective components and can be interpreted as shape components (Wiley, 1981). Head size and shape (HW and HL) loaded heavily on component 2, which accounted for 17% of the variation in the data. Component 3 was related to the width of three structures (CW, FWW and HWW); this component accounted for 9% of the variation in the data. Finally, component 4 was also related to the width of three structures (HW, TW and BW) and accounted for 6% of the total variance in the data.

Colony component scores were calculated using each character's coefficients associated with each component and the means for each population were compared by ANOVA. There were significant differences amongst both populations for the 4 components (Tab. III). The population from the Yucatan Peninsula had a significantly smaller mean for component 1,

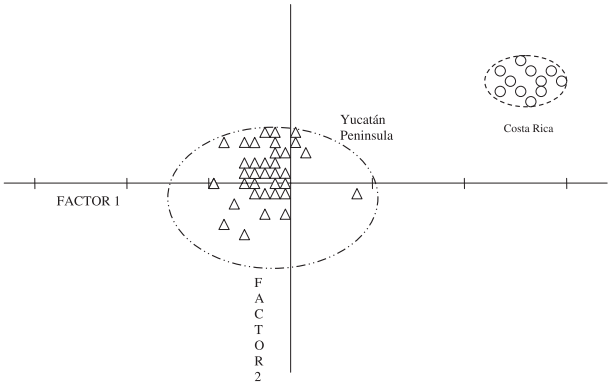

Figure 3. Distribution of PCA scores of *M. beecheii* colonies from the Yucatan Peninsula and Costa Rica against components 1 and 2.

Table IV. Summary of genetic information for populations of *M. beecheii* from the Yucatan Peninsula and Costa Rica, giving the effective number of alleles per population (N_a), observed (H_o) and expected (H_e) heterozygosity, the inbreeding coefficient (F_{IS}) and F_{ST}, a measure of population differentiation. Means ± SE are given for values across all loci.

	N_a		H_o		H_e		F_{IS}		F_{ST}
Locus	Yucatan Peninsula	Costa Rica	Yucatan Peninsula	Costa Rica	Yucatan Peninsula	Costa Rica	Yucatan Peninsula	Costa Rica	
T4	2.0	1.81	0.333	0.230	0.848	0.563	0.607*	0.591*	0.385
T7	2.20	1.89	0.468	0.461	0.547	0.655	0.144*	0.296*	0.467
Mbi11	1.97	1.91	0.478	0.308	0.499	0.694	0.042	0.556*	0.177
Mbi201	1.92	1.95	0.405	0.454	0.488	0.873	0.170*	0.479*	0.382
B116	7.33	4.51	0.859	0.909	0.938	0.951	0.084*	0.044	0.054
B124	1.65	1.62	0.301	0.250	0.361	0.532	0.166*	0.530*	0.074
overall	2.84 ± 2.20	2.28 ± 1.09	0.474 ± 0.201	0.435 ± 0.252	0.535 ± 0.226	0.711 ± 0.168	0.193* ± 0.207	0.416* ± 0.209	0.280 ± 0.080

* Significantly different from zero at $P < 0.05$.

related to body size, and for component 2, related to the size and shape of the head, compared to the population from Costa Rica ($P < 0.01$). The results for components 3 and 4 showed a similar pattern, with the population from the Yucatan Peninsula scoring smaller values compared with the population from Costa Rica. Finally, the orthogonal projection of PCAs against components 1 and 2 showed complete separation between the populations from the Yucatan Peninsula and Costa Rica (Fig. 3).

3.2. Molecular studies

The frequencies of alleles, the observed and expected heterozygosities per locus, the mean effective number of alleles per locus and population, and the overall summary data for each population are presented in Table IV. Both populations studied were polymorphic for the six microsatellite loci investigated. No pair of loci was significantly linked ($P > 0.05$).

The total number of alleles detected per locus ranged from 3 in loci T4 and B124 to 27 in locus B116. The effective number of alleles per population (observed allelic diversity) for the most variable locus, B116, ranged from 4.5 in the population from Costa Rica to 7.3 in the Yucatan Peninsula (Tab. IV). Average observed heterozygosities were 0.435 ± 0.25 in Costa Rica and 0.473 ± 0.2 in the Yucatan Peninsula. Overall expected heterozygosities were 0.711 ± 0.16 in the Costa Rican population and 0.535 ± 0.22 in the Yucatan Peninsula (Tab. IV).

Across all loci, there was a total of 32 private alleles, that is, alleles found in either

the Yucatan Peninsula (24 alleles) or Costa Rica (8 alleles) but not both. However, in the Yucatan Peninsula population 12 of the private alleles were found in locus B116 that was extremely polymorphic (27 alleles observed in this study). Moreover, allele frequencies differed greatly between the Yucatan Peninsula and Costa Rica. Single locus F_{ST} values ranged between 0.023 for locus B116 to 0.378 for locus T7 and were all different from zero ($P < 0.01$), indicating substantial genetic differentiation between populations. Multilocus F_{ST} was 0.280 ($P < 0.001$) which confirmed that populations of *M. beecheii* from the Yucatan Peninsula and Costa Rica were significantly different from each other at the molecular level as well as at the morphological level.

Exact tests revealed departure from Hardy-Weinberg equilibrium for all loci tested ($P < 0.001$) due to heterozygote deficit. The values of F_{IS} were also universally positive across loci and for both populations (Tab. IV), further indicating a lack of heterozygotes, except for locus Mbi11 in the Yucatan Peninsula population and for locus B116 for the Costa Rican one. This result was confirmed by the overall significant deviation from Hardy-Weinberg expectations across loci and populations (χ^2 = infinity; df 20).

COI (GenBank Accession numbers CR2 836923-ME2 836925) sequences were trivial to align. Of the 678 bp which were sequenced, those from Yucatan were identical, and the two from Costa Rica were likewise identical. However, there were 8 substitutions (1.2% sequence divergence) between Costa Rican and Yucatan Peninsula bees and 22 fixed differences out of 678 bp (3.2% sequence divergence) between *M. beecheii* and *M. bicolour*. Though we lack a robust phylogeny of the genus *Melipona* with which to interpret the intraspecific difference, our data suggest that the two populations show considerable divergence.

4. DISCUSSION

The results of both the morphometric and molecular analyses showed that populations of *M. beecheii* from Costa Rica and the Yucatan Peninsula exhibit substantial differentiation, and that both morphology and genetics concur in their signal of population genetic differentiation.

The first evidence for differentiation between the populations comes from integument colour. Body colour characters are commonly used in traditional taxonomy of bees to separate taxa (Schwarz, 1948; Pamilo et al., 1984; Ruttner, 1988; Waldschmidt et al., 2000). Schwarz (1932) even commented on the differences in colour forms of *M. beecheii* across its range. However, colour variation may not be reliable when used alone for classification purposes in bees (e.g. *B. terrestris,* see Estoup et al., 1996). Rather, we used face coloration as an additional characteristic to the analysis of morphometrics and microsatellites and found differences in the extent of yellow marks on the face of *M. beecheii.* Similar results were found when analysing *M. beecheii* populations from two regions in Mexico (Carrillo et al., 2001) where bees from southern Chiapas had smaller yellow marks on the clypeus and malar area compared to bees from the northern part of the Yucatan Peninsula.

In our study, bees from Costa Rica were also darker than bees from the Yucatan Peninsula. Costa Rican bees collectively lacked the extended yellow stripes on the clypeus compared with those from the Yucatan Peninsula. The evidence from this study and that from Carrillo et al. (2001) suggests that colour variability may be greater between populations of *M. beecheii* than within them, and that this pattern could be useful in establishing the geographic origin of specimens. More samples from Cuba, Central America and other areas of Mexico need to be analysed to support this hypothesis.

Body size also differed between Yucatan Peninsula and Costa Rican bees, the former being significantly smaller compared with their southern counterparts. Worker bee size may reflect adaptations to local environmental conditions (Ruttner, 1988). Hepburn and Radloff (1998) and Hepburn et al. (2001) have established empirically that the greater the geographic distances between honey bee populations, the greater the likelihood that such

groups will show substantial separation in multivariate analyses of size, though whether this is due to selection, genetic drift, or both is unclear. *Melipona beecheii* samples that we analysed came from two extreme distributional localities of the species so it is unsurprising that, as with colour, bees differed in body size. Whether this size difference is due to selection or drift or both, remains to be investigated.

The findings of differences in colour and size in *M. beecheii* hint at possible differentiation between populations at the genetic level too. Few molecular studies have been conducted on stingless bees to address their population genetics (Waldschmidt et al., 2000, 2002; Francisco et al., 2001; Castanheira and Contel, 2005). To our knowledge ours is the first one to include microsatellite loci. Microsatellites are valuable tools for population studies due to their high level of polymorphism as well as being selectively neutral and displaying codominance and Mendelian inheritance (Goldstein and Schlötterer, 1999).

The results of our microsatellite analysis revealed differentiation between Yucatan Peninsula and Costa Rican *M. beecheii*. The high values of F_{ST} confirmed that there were significant molecular genetic differences between the two populations. Such genetic heterogeneity between Yucatan and Costa Rica can be explained by reduced gene flow that leads to significant differentiation between populations.

Sequence divergence between Costa Rican and Yucatan Peninsula samples of *M. beecheii* at the COI gene was also marked (1.2%). This mitochondrial gene has become the candidate for DNA barcoding studies (e.g. Hebert et al., 2003a,b; Savolainen et al., 2005) and therefore there is a considerable number of sequences deposited in data banks (e.g. GenBank) for many insect species. Typically, COI sequence divergence between insect species is of the order of 3–10% (Hebert et al., 2003a,b; Monaghan et al., 2005), though sibling species may show far lower sequence divergence (down to 0.32%, Hebert et al., 2004). Among bee species, sibling species of *Colletes* show no fixed differences in COI sequences (Kuhlmann et al., 2007) whilst species of Euglossini may differ by < 1% (Dick et al., 2004). In contrast, cryptic species of *Bombus* differ by ca. 3% (Berstch et al., 2005) and the major geographic races of the honey bee *A. mellifera* differ by > 2% (Smith, 1991). It is therefore not yet possible to evaluate whether the observed divergence of 1.2% between populations of *M. beecheii* represents different species or intraspecific diversity until more thorough sampling of this and other stingless bee species has been undertaken.

In contrast to the situation in honey bees (Winston, 1987) and bumblebees (Widmer and Schmid-Hempel, 1999), stingless bee colonies and their female reproductives do not migrate and show only reduced dispersal (a few hundred metres at the most) during swarming (Engels and Imperatriz-Fonseca, 1990). Their particular mode of reproduction involves a gradual movement of construction and food materials between mother and daughter colonies and prevents long distance dispersal of their maternally inherited mitochondrial genes. Moreover, the existence of large areas devoid of primary forests necessary for nesting strongly affects species of *Melipona* that then become isolated in forest fragments (Brown and Albrecht, 2001).

We found reduced heterozygosity compared to expected values in both populations of *M. beecheii* for most microsatellite loci. There was also significant inbreeding in both populations (F_{IS} was positive and there was significant deviation from Hardy Weinberg equilibrium). At least for the Yucatan Peninsula, where feral populations of *M. beecheii* are extremely rare, deforestation (Gomez-Pompa and Kaus, 1999), human management (Quezada-Euán et al., 2001) and other environmental factors such as the use of pesticides (Valdovinos-Nuñez et al., 2003), interspecific competition with honey bees for food resources (Pinkus-Rendon et al., 2005) and nest sites (Santos-Leal, 2006) may act to reduce the species' population size and increase inbreeding. These factors may also impact populations of *M. beecheii* in Costa Rica and elsewhere.

Presently, the northern part of the Yucatan Peninsula is composed of a few patches of less-managed forests coexisting with vast areas of agroforestry systems and abandoned

agricultural land (Gomez-Pompa and Kaus, 1999). Habitat fragmentation may severely affect feral populations of *M. beecheii* from the Yucatan Peninsula by reducing colony dispersion and genetic drift (Carvalho et al., 1995). Our results provide information for current conservation programmes in the region and elsewhere. Efforts should aim to maintain genetic variability by promoting the reproduction of as many colonies as possible. Given the marked differentiation between populations from different regions that may even represent different species, caution needs to be exercised in the movement of colonies between locations occupied by this species in southern Mexico and Central America.

ACKNOWLEDGEMENTS

To CONACYT-SAGARPA, the IFS and the BBVA foundation for supporting this study. Judith Slaa and Koos Biesmeijer kindly provided the Costa Rican samples used in this study.

Les caractères morphologiques et moléculaires révèlent une différenciation chez l'abeille sociale néotropical *Melipona beecheii* (Apidae, Meliponini).

***Melipona* / abeille sans aiguillon / structure population / variabilité génétique / Yucatan / Costa-Rica**

Zusammenfassung – Morphologische und molekulare Marker weisen auf eine Differenzierung in Populationen der neotropischen Stachellosen Biene *Melipona beecheii* (Apidae : Meliponini) hin. Die über 400 Arten umfassenden pantropisch verbreiteten Meliponini sind vergleichsweise wenig untersucht, insbesondere gibt es nur wenige Studien zur Populationsstruktur. Die Art *Melipona beechei* weist eine geographische Verbreitung von Mexiko bis Costa Rica auf (Abb. 1), so dass es möglich ist, dass lokal adaptierte Ökotypen existieren. Wir nutzen einen morphometrischen Ansatz, sowie DNA Mikrosatellitenloci und Gensequenzen mitochondrialer DNA um die Populationsstruktur von *M. beecheii* an den Extrempunkten der geographischen Verbreitung zu untersuchen.
Die Bienen wurden 1998 an sieben Orten auf der Halbinsel Yucatan (65 Völker) und an einem Ort in Costa Rica (15 Völker) gesammelt. Elf Morphometriemerkmale am Kopf, an Flügeln und Beinen wurden an 10–12 Arbeiterinnen pro Volk vermessen und die einzelnen Standorte wurden mittels ANOVA (gefolgt von Tukey post hoc Tests) verglichen. Populationsunterschiede in Farbmarken am Kopf (Abb. 2) wurden mittels Kontingenz *G*-Test herausgearbeitet. Anschliessend wurde eine Hauptkomponentenanalyse (PCA auf der Basis einer Korrelationsmatrix) mit allen log-transformierten metrischen Merkmalen durchgeführt.
Sechs Mikrosatellitenloci (T4, T7, Mbi11, Mb201, B116 und B124) wurden an jeweils einer Arbeiterin pro Volk untersucht. Zusätzlich wurden 678 Basenpaare des mitochondrialen Gens COI für jeweils zwei Individuen aus Costa Rica und Yucatan sequenziert. Die Verknüpfung (linkage) der Mikrosatellitenloci wurde mittels des Programmpakets GENEPOP Version 3.1. getestet. Die Allelfrequenzen und beobachtete sowie Nei's (1978) unabhängige Erwartungswerte für Heterozygotie an jedem Mikrosatellitenlocus wurden für jede Population mittels des FSTAT Programmpakets ermittelt. Die effektive Anzahl an Allelen (n_a) wurde als $n_a = 1/\Sigma p_i^2$ berechnet, wobei p_i die Frequenz des i-ten Allels darstellt. Abweichungen vom Hardy-Weinberg-Gleichgewicht an jedem Locus und für jede Population wurden mittels in GENEPOP verfügbaren exakten Tests in einem Markov Ketten-Ansatz ermittelt. Der unabhängige Multilocus-Schätzwert für F_{ST} zwischen Populationen wurde benutzt, um die Signifikanz der genetischen Differenzierung mittels FSTAT herauszuarbeiten und um den jeweiligen Inzuchtgrad F_{IS} und seine Konfidenzintervalle zu berechnen.
Die Populationen aus Costa Rica und Yucatan zeigten eine erhebliche phänotypische und genetische Differenzierung. Dabei bildeten die Völker aus Yucatan eine homogene Gruppe, die sich deutlich (als kleiner und weniger farbkräftig) von den Costa Rica Völkern unterschied (Tab. I, II; Abb. 3). Der Multilocus Wert für $F_{ST} = 0{,}280$ ($P < 0{,}001$) belegt die signifikante molekulargenetische Differenzierung zwischen den beiden Populationen (Tab. IV). Beide Populationen zeigten erhebliche Abweichungen vom Hardy-Weinberg Gleichgewicht. Die F_{IS}-Werte für Costa Rica = 0,416 ($P < 0{,}05$) und Yucatan = 0,193 ($P < 0{,}05$) waren hochsignifikant, was auf ein vermutlich inzuchtbedingtes Fehlen an Heterozygoten hinweist. In den DNA-Sequenzen für COI unterschieden sich die beiden Populationen um 1,2 %. Die Ergebnisse der vorliegenden Studie sollten in Konservierungsprogramme Eingang finden, vor allem dann, wenn der Austausch von Völkern zwischen Regionen in Betracht gezogen wird.

Stachellose Bienen / *Melipona* / Populationsanalyse / Yucatan / Costa Rica / Genetische Diversität

REFERENCES

Ayala R. (1999) Revisión de las abejas sin aguijón de México (Hymenoptera: Apidae: Meliponini), Folia Entomol. Mex. 106, 1–123.

Berstch A., Schweer H., Titze A., Tanaka H. (2005) Male labial gland secretions and mitochondrial DNA markers support species status of *Bombus cryptarum* and *B. magnus* (Hymenoptera, Apidae), Insectes Soc. 52, 45–54.

Biesmeijer J.C. (1997) The organisation of foraging in stingless bees of the genus Melipona, Ph.D. Thesis, Universitat Utrecht.

Brown J.C., Albrecht C. (2001) The effect of tropical deforestation on stingless bees of the genus *Melipona* (Insecta: Hymenoptera: Apidae: Meliponini) in central Rondonia, Brazil. J. Biogeogr. 28, 623–634.

Camargo J.M.F., Moure J.S., Roubik D.W. (1988) *Melipona yucatanica* new species (Hymenoptera: Apidae; Meliponinae); stingless bee dispersal across the Caribbean arc and Post-Eocene vicariance, Pan-Pacific Entomol. 64, 147–157.

Carrillo A., Quezada-Euán J.J.G., Moo-Valle J.H. (2001) Estudio preliminar sobre la variabilidad morfológica de *Melipona beecheii* (Apidae: Meliponini) en su rango de distribución de México, América Central y el Caribe, in: Quezada-Euán J.J.G., May-Itzá W. de J., Moo-Valle H., Chab-Medina J.C. (Eds.), II Seminario Mexicano sobre abejas sin aguijón, Mérida Yucatán, México, pp. 73–78.

Carvalho G.A., Kerr W.E., Nascimento V.A. (1995) Sex determination in bees. XXXVII. Decrease of Xo heteroalleles in a finite population of *Melipona scutellaris*, (Apidae, Meliponini), Brazil. J. Genet. 18, 13–16.

Castanheira E.B., Contel E.P.B. (2005) Geographic variation in *Tetragonisca angustula* (Hymenoptera, Apidae, Meliponinane), Apic. Res. 44, 101–105.

Dick C.W., Roubik D.W., Gruber K.F., Bermingham E. (2004) Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae, Euglossini) revealed by comparative mitochondrial DNA phylogeography, Mol. Ecol. 13, 3775–3785.

Diniz-Filho J.A., Pignata M.I.B. (1994) Quantitative genetics of multivariate morphometric variation in the neotropical stingless bee *Scaptotrigona postica* (Hymenoptera: Meliponinae), Rev. Bras. Genet. 17, 259–265.

Engels W., Imperatriz-Fonseca V.L. (1990) Caste development, reproductive strategies and control of fertility in honeybees and stingless bees, in: Engels W. (Ed.), Social Insects: an evolutionary approach to castes and reproduction, Berlin, Springer-Verlag, pp. 166–230.

Estoup A., Solignac M., Harry M., Cornuet J.M. (1993) Characterization of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris,* Nucleic Acid Res. 21, 1427–1431.

Estoup A., Garnery L., Solignac M., Cornuet J.M. (1995) Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models, Genetics 140, 679–695.

Estoup A., Solignac M., Cornuet J.M., Goudet J., Scholl A. (1996) Genetic differentiation of island and continental populations of *Bombus terrestris* (Hymenoptera:Apidae) in Europe, Mol. Ecol. 5, 19–31.

Francisco F.O., Silvestre D., Arias M.C. (2001) Mitochondrial DNA characterization of five species of *Plebeia* (Apidae: Meliponinae) RFLP and restriction maps, Apidologie 32, 323–332.

Franck P., Garnery L., Loiseau A., Oldroyd B.P., Hepburn H.R., Solignac M., Cornuet J.M. (2001) Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data, Heredity 86, 420–430.

Goldstein D.B., Schlötterer C. (1999) Microsatellites: evolution and applications, Oxford Univ. Press. Inc., New York.

Gomez-Pompa A., Kaus A. (1999) From pre-Hispanic to future conservation alternatives: lessons from Mexico, Proc. Natl. Acad. Sci. (USA) 96, 5982–5986.

Goudet J. (1996) FSTAT (vers. 1.2): a computer program to calculate F-statistics, Heredity 86, 485–486.

Guo S.W., Thompson E.A. (1992) Performing the exact test of Hardy-Weinberg proportion of multiple alleles, Biometrics 48, 361–372.

Hartfelder K., Engels W. (1992) Allometric and multivariate analysis of sex and caste polymorphism in the neotropical stingless bee, *Scaptotrigona postic.*, Insectes Soc. 39, 251–266.

Hebert P.D.N., Cywinska A., Ball S.L., de Waard J.R. (2003a) Biological identifications through DNA barcodes, Proc. R. Soc. Lond. B 270, 313–321.

Hebert P.D.N., Ratnasingham S., de Waard J.R. (2003b) Barcoding animal life: cytochrome *c*oxidase subunit I divergences among closely related species, Proc. R. Soc. Lond. B (Suppl.) 270, S96–S99.

Hebert P.D.N., Penton E.N., Burns J.M., Janzen D.H., Hallwachs W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*, Proc. Natl. Acad. Sci. (USA) 101, 14182–14187.

Hepburn H.R., Radloff S.E. (1998) Honeybees of Africa, Springer Verlag, Berlin.

Hepburn H.R., Radloff S.E., Verma S., Verma L.R. (2001) Morphometric analysis of *Apis cerana*

populations in the southern Himalayan region, Apidologie 32, 435–447.

Hepburn H.R., Radloff S.E., Otis G.W., Fuchs E., Verma L.R., Ken T., Chaiyawong T., Tahmasebi G., Ebadi R., Wongsiri S. (2005) *Apis florea*: morphometrics, classification and biogeography, Apidologie 36, 359–376.

Kerr W.E. (2002) Extincao de especies: a grande crise biologica do momento e como afeta os meliponinios. Anais do V encontro sobre abelhas, 2002, Riberao Preto, SP Brasil, pp. 4–9.

Kuhlmann M., Else G.R., Dawson A., Quick D.L.J. (2007) Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae), Org. Divers. Evol. (in press).

Michener C.D. (2000) The bees of the world. The Johns Hopkins Univ. Press, Baltimore.

Monaghan M.T., Balke M., Gregory T.R., Vogler A.P. (2005) DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers, Phil. Trans. R. Soc. Lond. B 360, 1925–1933.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals, Genetics 89, 583–590.

Pamilo P., Varvio-Aho S.L., Pekkarinen A. (1984) Genetic variation in bumblebees (*Bombus, Psithyrus*) and putative sibling species of *Bombus lucorum*, Hereditas 101, 245–251.

Paxton R.J., Wei*β*schuh N., Quezada-Euán J.J.G. (1999) Characterization of dinucleotide microsatellite loci for stingless bees, Mol. Ecol. 8, 93–99.

Peters J.M., Queller D.C., Imperatriz-Fonseca V.L., Roubik D.W., Strassman J.E. (1999) Mate number, kin selection and social conflicts in stingless bees and honeybees, Proc. R. Soc. Lond. B 266, 379–384.

Pinkus-Rendon M.A., Parra-Tabla V., Meléndez-Ramírez V. (2005) Floral resource use and interactions between *Apis mellifera* and native bees in cucurbit crops in Yucatán, México, Can. Entomol. 137, 441–449.

Quezada-Euán J.J.G., May-Itza W. de J., Gonzalez-Acereto J.A. (2001) Meliponiculture in México: problems and perspective for development, Bee World 82, 160–167.

Radloff S.E., Hepburn H.R., Fuchs E., Otis G.W., Hadisoesilo S., Hepburn C., Ken T., (2005) Multivariate morphometric analysis of the *Apis cerana* populations of oceanic Asia, Apidologie 36, 359–376.

Raymond M., Rousset F. (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism, J. Heredity 86, 248–250.

Roubik D.W. (1989) Ecology and natural history of tropical bees, Cambridge University Press.

Ruttner F. (1988) Biogeography and taxonomy of honeybees, Springer Verlag, Berlin.

Santos-Leal A. (2006) Distribución espacial de los sitios de anidación de abejas eusociales (Hymenoptera-Apidae: Meliponini y Apini) en Sudzal, Yucatán, México, Tesis de Maestria, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, México.

Savolainen V., Cowan R.S., Vogler A.P., Roderick G.K., Lane R. (2005) Towards writing the encyclopedia of life: and introduction to DNA barcoding, Phil. Trans. R. Soc. Lond. B 360, 1805–1811.

Schwarz F.H. (1932) The genus *Melipona*. The type genus of the Meliponidae or stingless bees, Bull. Am. Mus. Nat. Hist. 63, 231–460

Schwarz F.H. (1948) Stingless bees (Meliponidae) of the Western Hemisphere, Bull. Am. Mus. Nat. Hist. 90, 1–546.

Simon C., Frati F., Beckenback A., Crespi B., Liu H., Flook P. (1994) Evolution, weighing and phylogenetic utility of mitochondrial DNA sequences and a compilation of conserved polymerase chain reaction primers, Ann. Entomol. Soc. Am. 87, 651–701.

Smith D.R. (1991) African bees in the Americas: insights from biogeography and genetics, Trend. Ecol. Evol. 6, 17–21.

Staden R., Beal K.F., Bonfield J.K. (2000) The Staden package, 1998, Methods in Mol. Biol. 132, 115–130.

Thompson J.D., Higgins D.G., Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressing multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22, 4673–4680.

Valdovinos-Nuñez G.R., Quezada-Euán J.J.G., Marrufo-Olivares J. (2003) Efecto de la aplicación aérea de permetrina en *Apis mellifera* y abejas nativas sin aguijón (Hymenoptera: Apidae) en Yucatán, México, XVII Seminario Americano de Apicultura, Aguascalientes, México, pp. 147–149.

Waldschmidt A.M., Barros E.G., Campos L.A.O. (2000) A molecular marker distinguishes the subspecies *Melipona quadrifasciata quadrifasciata* and *Melipona quadrifasciata anthioides* (Hymenoptera: Apidae, Meliponinae), Gen. Mol. Biol. 23, 609–611.

Waldschmidt A.M., Marco-Junior P., Barros E.G., Campos L.A.O. (2002) Genetic analysis of *Melipona quadrifasciata* Lep. (Hymenoptera: Apidae: Meliponinae) with RAPD markers, Braz. J. Biol. 62, 923–928.

Weir B.S., Cockerham C.C. (1984) Estimating *F*−statistics for the analysis of population structure, Evolution 38, 1358–1370.

Widmer A., Schmid-Hempel P. (1999) The population genetic structure of a large temperate pollinator species, *Bombus pascuorum* (Scopoli) (Hymenoptera: Apidae), Mol. Ecol. 8, 387–398.

Widmer A., Schmid-Hempel P., Estoup A., Scholls A. (1998) Population genetic structure and colonization history of *Bombus terrestris* s.I. (Hymenoptera: Apidae) from the Canary Islands and Madeira, Heredity 81, 563–572.

Wiley E.O. (1981) Phylogenetics, John Wiley and Sons, New York.

Winston M.L. (1987) The biology of the honey bee, Harvard Univ. Press, Cambridge, Massachusetts.